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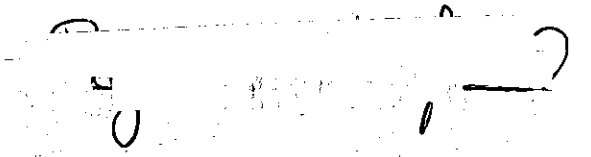

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OBSERVATIONS ON THE CHROMOSOMAL COMPLEMENTS OF CHINESE HAMSTER PRIMARY CELL STRAINS *IN VITRO*

A THESIS

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The Faculty of the Graduate Division

by

Eugene Allen Hatfield

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SUMMARY

The frequencies of chromosomal and mitotic aberrations were investigated in primary cell strains of the Chinese hamster, Cricetulus griseus. Frequencies of aneuploidy and euploidy were determined at metaphase. The frequency of anaphase cells having single and double fragments, single and double bridges, and multipolarity was determined. Data of this investigation were compared with data reported in the literature on Chinese hamster and other mammalian primary cell cultures.

The frequency of aneuploidy and polyploidy did not vary significantly during the first 30 days in vitro. The frequency of abnormal anaphases per cell was found to increase during the 30 day period due to a significant increase in fragments per cell at the 19-day analysis. The level of abnormalities present in primary cultures of Chinese hamster is low, although higher than that found in cultures of normal human tissue. The low frequency of spontaneous aberrations and excellent cytogenetic properties of primary cultures of Cricetulus griseus render this animal to be of great value for future cytogenetic research.

CHAPTER I

INTRODUCTION

Since the advent of improved cell culture techniques, many kinds of *in vitro* mammalian cell types have been analyzed cytogenetically. Cells that have been maintained *in vitro* for varying periods of time often show changes in chromosome morphology, ploidy, or cell type (Hsu, 1961; Levan and Biesele, 1958; Hayflick, 1965). Recent research done on mammalian cells *in vivo* has shown that the chromosomal abnormality level is near zero (Bender and Gooch, 1961). The purpose of this investigation is to observe the numerical and morphological sequence of cytological variations which occur in cells cultured from embryos of the Chinese hamster (*Cricetulus griseus*). It is important to establish a "baseline", or control, curve of aberrations. This is necessary if this cell type is to be used for studies on radiation effects, metabolic needs, or characteristics of transformation.

The first mammalian cell type widely cultured was the HeLa strain of Gey *et al.* (1952) isolated from human neoplastic tissue. Extensive research has been done using the HeLa line and other adapted cell lines of both normal and neoplastic origin. Cytogenetic studies of these cell lines have revealed that most cultured malignant cell lines have an abnormal karyotype which presumably results from failures of cytokinesis, mitotic abnormalities, endoreduplication, and chromosome breaks (Hsu *et al.*, 1961; Chu, 1962; Ford *et al.*, 1959; Hsu and Klatt, 1958; Chu and Giles, 1958; Moorhead and Hsu, 1956; and others). Improved techniques soon led

to the culture of human and other mammalian cells from normal tissue for extended periods of time and it was established that the karyotype of these cells did not vary significantly from the in vivo karyotype so long as the cell strain was actively dividing, known as Phase II of the cell growth curve (Hayflick, 1965; Puck et al., 1958; Tjio and Puck, 1958; Bender and Gooch, 1962; Norris and Hood, 1962; and others). This phase usually terminates after approximately 50 transfers in normal human primary cells and the cells enter Phase III, the terminal period, during which time the intervals between population doublings become progressively greater (Hayflick, 1965). Mouse cells have been extensively cultured and, cytogenetically, were soon found to be abnormal. This transformation from normal karyotype to abnormal karyotype was studied closely since the resulting cell lines were found to resemble the adapted human cultures and in vivo mouse neoplasms (Levan and Biesele, 1958; Chu et al., 1958; Rothfels and Parker, 1959; Hsu et al., 1961; and others). Injections of such transformed mouse strains produced tumors in inbred mouse strains. However, other research has suggested that an abnormal karyotype resulting from in vitro maintenance does not necessarily result in malignancy (Barski and Cassingena, 1963).

There now appear to two differing cell types with respect to stability in culture. Stability in this investigation is taken to mean conformation to the "baseline" established by in vivo studies, i.e. - normal chromosome morphology, and near zero frequency of spontaneous aberrations and aneuploidy. Those cells which show a lack of stability in vitro include mouse, rhesus monkey, and Chinese hamster cells (Levan and Biesele, 1958, and others; Kleinfeld and Melnick, 1958; Hsu, 1962; Hsu and Zenzes, 1964; Ford et al., 1959; Ford et al., 1961; and others).

Those cell types showing stability in culture include human, oppossum, and pig primary cells (Tjio and Puck, 1958; Hayflick, 1965; Ruddle, 1961; and others). Care must be taken before assuming a cell strain is "normal". Maddox (1967) investigated a "normal" strain of cells of the Tasmanian rat-kangaroo (Potorous tridactylus) lacking one chromosome and found considerable variation in relative length of chromosomes, centromere index, and arm ratio, as well as variation in the number of chromosomes present in the karyotype.

The lack of stability on the part of mouse, monkey, and Chinese hamster cells in vitro is shown by high spontaneous aberration frequencies after only short periods in culture resulting in aneuploidy, abnormal chromosomes, and finally heteroploidy (ex.: Hsu and Zenzes, 1964; Levan and Biesele, 1958). Such high frequencies of spontaneous aberrations have not been reported in primary human and other mammalian primary cell types of normal origin (Hayflick and Moorhead, 1961; Hayflick, 1965; Tjio and Puck, 1958). Once the diploid human cells reach Phase III of growth curve, however, the frequency of spontaneous aberrations in diploid human cells begins to increase until either the cell strain dies or transformation to an adapted cell line occurs (Hayflick and Moorhead, 1961; Hayflick, 1965; Hayflick, 1968). Transformation is defined as a change from a diploid chromosomal complement with a finite growth period to a heteroploid, neoplastic-like, chromosomally unstable cell line (Hayflick, 1965; Sanford, 1967; and others). This transformation occurs most readily in mouse cells, but Chinese hamster cells are also prone to transformation. Human cells have rarely been reported to become transformed (Van Scott et al., 1965). However, because of the difficulties

of analysis of the human karyotype, few detailed studies have been performed.

Successful laboratory breeding of the Chinese hamster (Cricetulus griseus) has provided an excellent source of cells for cytogenetic investigations including the connections which may exist between cancer and the chromosome variations which occur in vitro due to transformation, age in culture, radiation, and some viruses. The Chinese hamster has 22 chromosomes: 11 pairs which are morphologically distinct (Yerganian, 1952 and 1958). This makes them more useful for cytogenetic investigation than human or mouse cells.

Puck et al. (1958) suggested that a stemline of cells existed in Chinese hamster cell lines which had 22 chromosomes; however, they also found a significant proportion of cells containing 21 and 23 chromosomes. Ford and Yerganian (1958) found the cells retaining diploidy early in in vitro life with a change to heteroploidy later. They also found that the cells exhibited several exchanges which resulted in abnormal karyotypes. Ford (1958) and Ford et al. (1959) continued the above research and confirmed that Chinese hamster primary cells remained diploid for varying periods up to several months in vitro. This research also indicated that the chromosomes retained their identity so that abnormal chromosomes could be traced to some breakage, exchange, or centromeric fracture. Ford et al. (1961) continued to investigate the response of Chinese hamster cells to the in vitro environment and found that there was a large amount of variability between cell strains, between sublines of the same strain, and between successive preparations of the same subline. This led to attempts to clone a diploid cell strain. Yerganian

and Leonard (1961) found that cell strain FAF-28 retained its diploid complement with very few spontaneous breaks. The cell strain had aneuploidy of less than 20 percent and tetraploidy of between 1 and 25 percent, generally due to endoreduplication. The cell strain was not subjected to detailed analysis of arm ratios or centromeric index however. A possible reason for the apparent stability of this cell strain was the extremely rapid proliferative rate which did not allow new stemline cells to gain a foothold. Hsu (1962) demonstrated a large amount of spontaneous change in two other cell strains of the Chinese hamster and showed that the age of the stock culture influenced the total amount of breakage, the older the stock culture, the larger the amount of breakage. Hsu and Zenzes (1964) attempted to find a diploid stemline cell and succeeded. This strain was designated DON. The DON cell line showed diploidy, but also was found to have many pseudodiploid cells with a considerable amount of exchange occurring. Stubblefield (1966) continued to clone the DON line and produced the DON-C line which apparently has the distal third of the short arm of autosome 1b translocated to the Y chromosome. This cell strain has been found to be numerically stable. However, numerical stability does not assure that a considerable amount of exchange and translocation is not occurring.

Bender and Gooch (1961) conducted studies on the radiation sensitivity of in vivo cells of the Chinese hamster. This experiment confirmed their earlier data on Ateles that the in vivo spontaneous aberration frequency was zero. They did not find one abnormal chromosome in the bone marrow preparations. More recent studies with human blood cultures have confirmed that humans apparently have no in vivo aberrations

unless exposed to radiation (Bender and Gooch, 1962) or due to increased age (Jacobs et al., 1961). However, work has been done which shows that neoplasms in humans often show a high frequency of spontaneous breakage in vivo (Fraccaro et al., 1965; Sandberg et al., 1961). This raises the question of how soon and why the karyotype of normal primary cells begins to change upon introduction to culture.

Sax and Passano (1961) demonstrated that the frequency of spontaneous aberrations increased in a primary human culture as the age of the culture increased. Hayflick (1965 and 1968) has confirmed this in human cells. Hsu (1962) also stated this occurred in Chinese hamster cells in vitro. This corresponds to the appearance of abnormal chromosome complements in vivo with increasing age in man (Jacobs et al., 1961) and mouse (Crowley and Curtis, 1963).

Due to the many effects of in vitro culture on chromosome breakage, it is desirable to study the response of primary cells from explant into culture throughout the first 30 days, or first few transfers, in order to establish more closely the changes which occur. Due to the excellent cytogenetic properties and availability, the Chinese hamster was chosen to provide the primary cultures.

CHAPTER II

MATERIALS AND METHODS

Source of Material

Primary cells of Chinese hamsters (Cricetulus griseus) were obtained from ten-day embryos removed from a female bred with male litter-mates. The pregnant female was sacrificed, embryos removed, and then minced mechanically with a surgical knife while observing aseptic techniques. The minced tissue was washed in growth medium and placed in prescription bottles.

Cells were grown in a medium composed of Eagle's minimal essential amino acids for monolayer culture with Eagle's non-essential amino acids (Eagle, 1959) in Hanks' saline solution. Twenty percent fetal calf serum with penicillin and streptomycin at 50 milligrams per liter were also added. The cell cultures were grown in prescription bottles in an incubator maintained at 35°C.

Slide Preparation

Cells to be analyzed were grown in wide-mouthed French square bottles into which microscope slides were placed. The cells were allowed to grow in the prescription bottles until a sufficient growth was obtained, usually less than one week. Following removal of excess tissue which failed to attach to the glass, the cell layer was scraped off with a silicone policeman. Growth medium was then mixed with the cell suspension and agitated. At each transfer, the cells were divided three ways:

one prescription bottle for continuing the culture and two bottles containing slides for metaphase and anaphase analysis. Cells were allowed to grow three to four days until a uniform cell layer had covered the slide. Cell growth was observed by using an inverted microscope and, following sufficient growth, slides were processed for analysis. At this time, the cells in the prescription bottle were transferred for the next analysis.

Cells to be analyzed at metaphase were arrested for five hours by introducing sufficient colchicine to give a final concentration of two milligrams per liter, placed in hypotonic solution at 35°C for twenty minutes, and then fixed by immersion into a solution made of three parts ethanol and one part acetic acid. The slides were fixed for several hours. The Coplin jar containing slide and fixative was then chilled for five minutes in a freezer of dry-ice, the excess fixative drained and the slide flamed to remove the remaining solution. It has been found that flaming flattens metaphase cells and permits more accurate analysis. The cooling minimizes cell distortion. These slides can be stored for long periods of time.

When ready for analysis, the slide was stained with proprionic-orcein. To assure good staining properties, the stain was prepared fresh each day as needed. Fifty percent proprionic acid solution was saturated with orcein and then boiled thoroughly, cooled and filtered to remove suspended solids. After placing a drop of stain on the slide, a coverslip was added and excess stain blotted away. The coverslip was then sealed with printer's wax to prevent drying out of the slide preparation.

Slides to be analyzed during anaphase were prepared in the same way except that the colchicine and hypotonic treatments were omitted. Thus the anaphase slides contained cells in all stages of mitosis.

Stained slides made using this method may be kept several months depending upon the care taken to seal the coverslip. Once the stain dries, the slides are not suitable for further detailed analysis. This method is modified from Ford and Hamerton (1956).

Analysis

All analyses in this study were made with phase contrast optics under 100X oil immersion.

In metaphase analysis for determination of chromosome numbers, all cells, which were flat with minimum chromosome overlap and apparently intact cell membrane were analyzed. Only those cells from which exact counts could be obtained were counted.

For polyploidy estimates, cells were analyzed at metaphase by counting cells with approximately twenty-two chromosomes as diploid, cells with approximately thirty-three chromosomes as triploid, and cells with approximately forty-four chromosomes as tetraploid. Cells with a larger number were counted as polyploid. All cells in which the chromosome number could be visualized were analyzed.

For analysis of anaphase cells, only cells in which the set of chromosomes was well separated were counted. This would tend to eliminate lagging chromosomes being counted as fragments or bridges. Cells were analyzed in anaphase and early telophase, or until apparent completion of cytokinesis.

CHAPTER III

RESULTS

One of the characteristics generally established when analyzing a new cell strain is that of the frequency of aneuploidy. One hundred metaphase cells were analyzed after four, eight, eleven, fifteen, and nineteen days *in vitro*. The amount of aneuploidy increased slightly but not significantly from the fourth day (89 percent diploid) to the nineteenth day (85 percent diploid). This was not a linear increase as may be seen in Figure 1 and Table 1. These results indicate a slight shift with increased age in culture from a higher percentage of hypodiploid cells to a higher percentage of hyperdiploid cells.

A second important characteristic is the percentage of polyploidy occurring in the culture. Figure 2 and Table 2 reveal that there was no significant change in the percentage of polyploid cells throughout the period of investigation. At least 100 metaphase cells were analyzed at each transfer. The percentage of cells having approximately a 3N, or triploid, number of chromosomes remained at one to two percent throughout the study. The 4N, or tetraploid, cells varied in frequency from 2.5 percent at the fifteenth day to 4.4 percent at the eighth day. These observations failed to show a significant change in the amount of ploy-ploidy during the period under investigation.

Analysis for frequency of abnormal anaphase is a third characteristic often used to study a cell strain. Here the analysis is broken down into three parts: abnormal anaphases per cell, fragments (single and

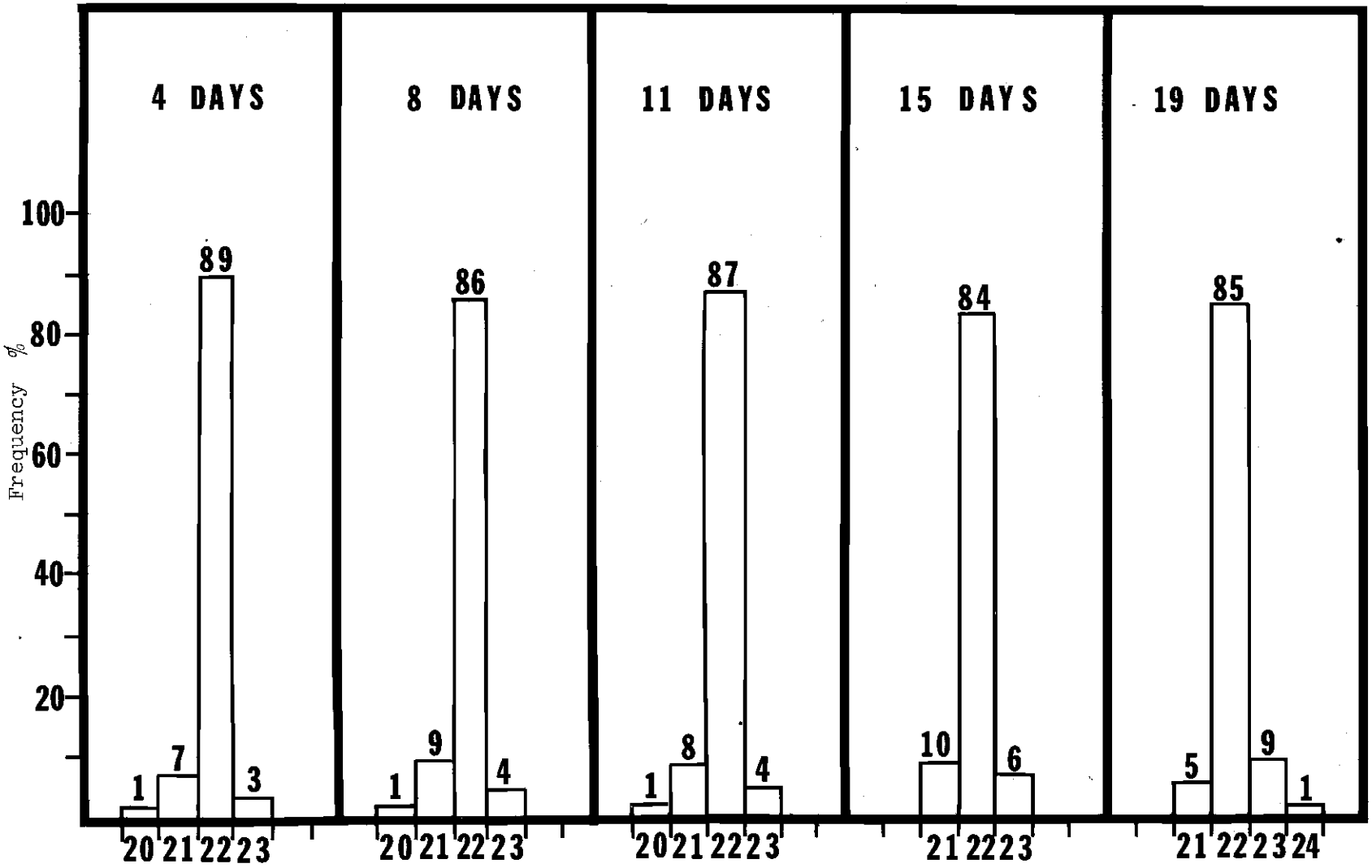

Figure 1. Frequency of Cells Having Exact Chromosome Number of 20 to 24 Chromosomes per Cell.

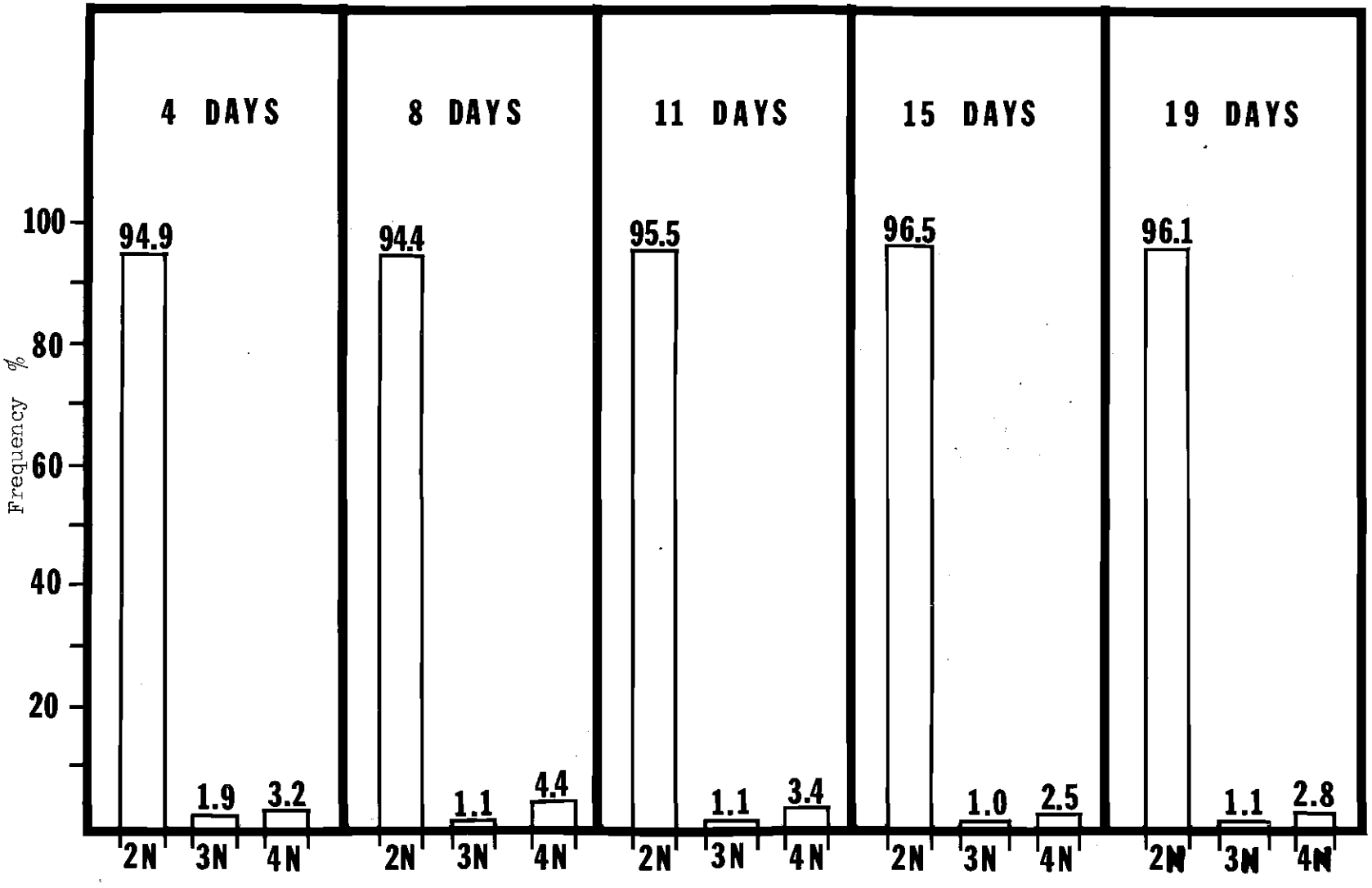

Figure 2. Frequency of Cells Having Approximately 2N, 3N or 4N Chromosomes per Cell Counted at Metaphase.

Table 1. Analysis of Metaphase Cells with Countable Complement for Frequency of Aneuploidy.

Days	Total Cell No.	20	21	22	23	24
4	100	1	7	89	3	0
8	100	1	9	86	4	0
11	100	1	8	87	4	0
15	100	0	10	84	6	0
19	100	0	5	85	9	1

Table 2. Analysis of Metaphase Cells with Visualizable Complement for Frequency of Polyploidy and Diploidy.

Days	Total Cell No.	2N	3N	4N	+N
4	158	150	3	5	0
8	180	170	2	8	0
11	179	171	2	6	0
15	119	115	1	3	0
19	179	172	2	5	0

double) per cell, and bridges (single and double) per cell. Table 3 presents the results of the overall anaphase analysis. In this investigation, a cell would be considered normal if it were undergoing dipolar division without chromosome fragments or bridges. As mentioned previously, anaphase when used for analysis of the karyotype does not permit determination of exchanges and other chromosome changes; however, it is adequate for comparative purposes. From Table 3, it can be seen that the percentage of cells undergoing normal division varied around the 90 percent frequency. There was no apparent change in the percentage of normal cells over the first fifteen days, with the average being 93 percent. However, the analysis after nineteen days revealed a slight decrease in the percentage from 92.1 percent (4 days) to 89.8 percent (19 days) with the third analysis (11 days) having 93.1 percent normal divisions. The number of abnormal dipolar divisions showed the same relationship. The frequency of multipolar divisions remained essentially stable, varying from 0.8 percent (11 days) to 1.7 percent (8 days) with the average for the period of study being 1.4 percent. Only two cells were found undergoing tetrapolar division. The remainder consisted of tripolar divisions. The frequency of tripolar divisions (1.4 percent) agrees well with the percentage of triploid cells (1.0 percent to 1.9 percent) found at metaphase. The frequencies of deletions and bridges are not necessarily the sum of the frequency of abnormal dipolar anaphases because some cells possessed both a bridge and a deletion.

Detailed analysis of abnormal dipolar divisions was undertaken to ascertain the major contributor to the abnormalities. Figure 3 and Table 4 represent the sum of both fragments and bridges per cell. There

Table 3. Composite of Anaphase Analysis, Including all Abnormalities Investigated.

Days	Total Cells Analyzed	Normal*	Abnormal Dipolar	Multi-polar	Deletions Single	Deletions Double	Bridges Single	Bridges Double
4	742	683	49	10	31	15	13	1
	100%	92.1%	6.6%	1.3%	4.2%	2.0%	1.8%	0.1%
8	636	596	29	11	17	6	10	0
	100%	93.7%	4.6%	1.7%	2.7%	0.9%	1.6%	0%
11	653	608	40	5	24	7	12	1
	100%	93.1%	6.1%	0.8%	3.7%	1.1%	1.8%	0.2%
15	489	451	33	5	17	5	15	1
	100%	92.2%	6.7%	1.0%	3.5%	1.0%	3.0%	0.2%
19	508	456	45	7	29	12	11	0
	100%	89.8%	8.8%	1.4%	5.7%	2.4%	2.2%	0%
23	200	180	16	4	13	7	1	0
	100%	90%	8%	2%	6.5%	3.5%	0.5%	0%

* See text for criteria of a normal cell.

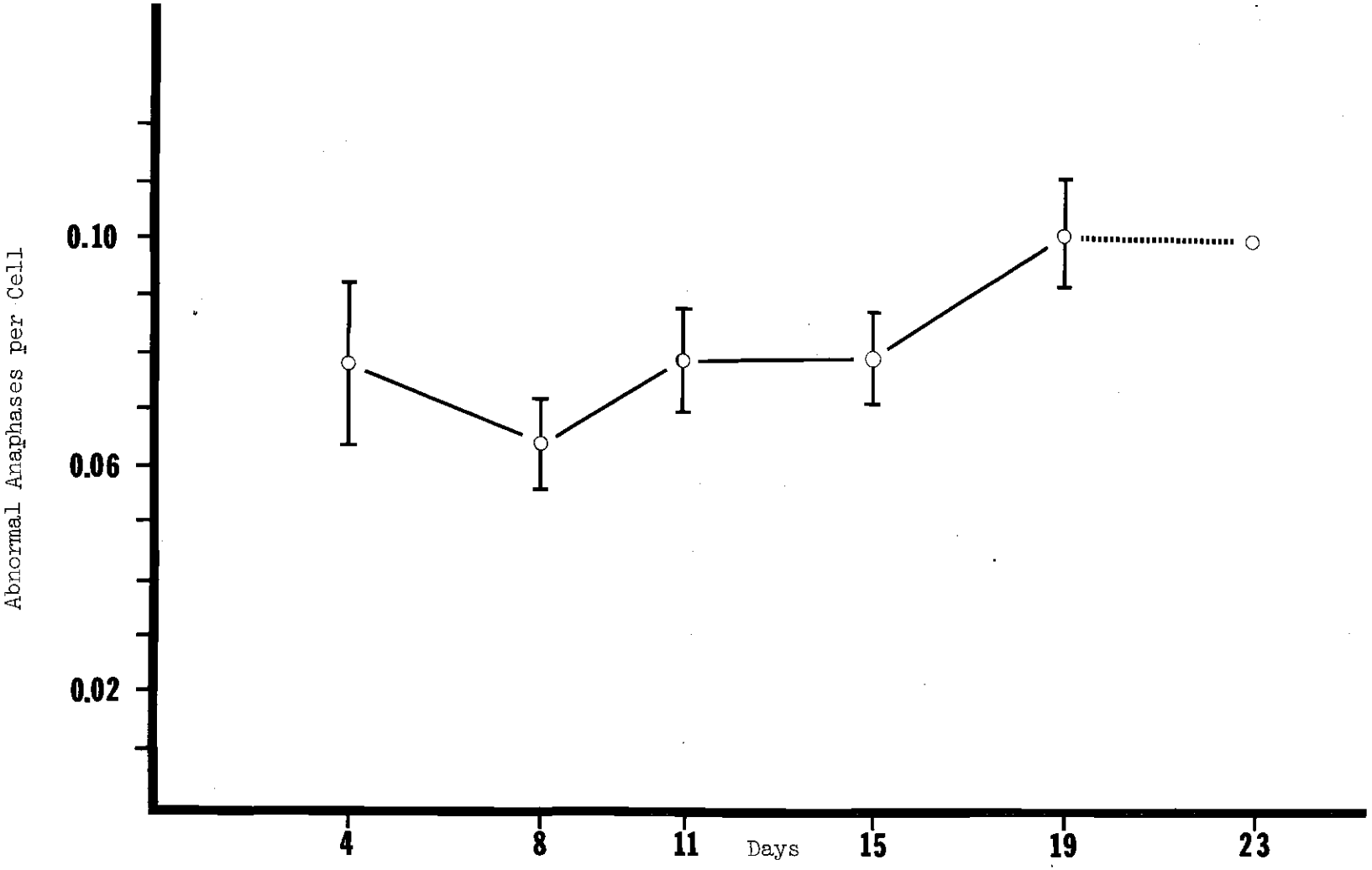

Figure 3. Frequency of Abnormal Anaphases per Cell as a Function of Time in Culture With Standard Error of the Mean Indicated.

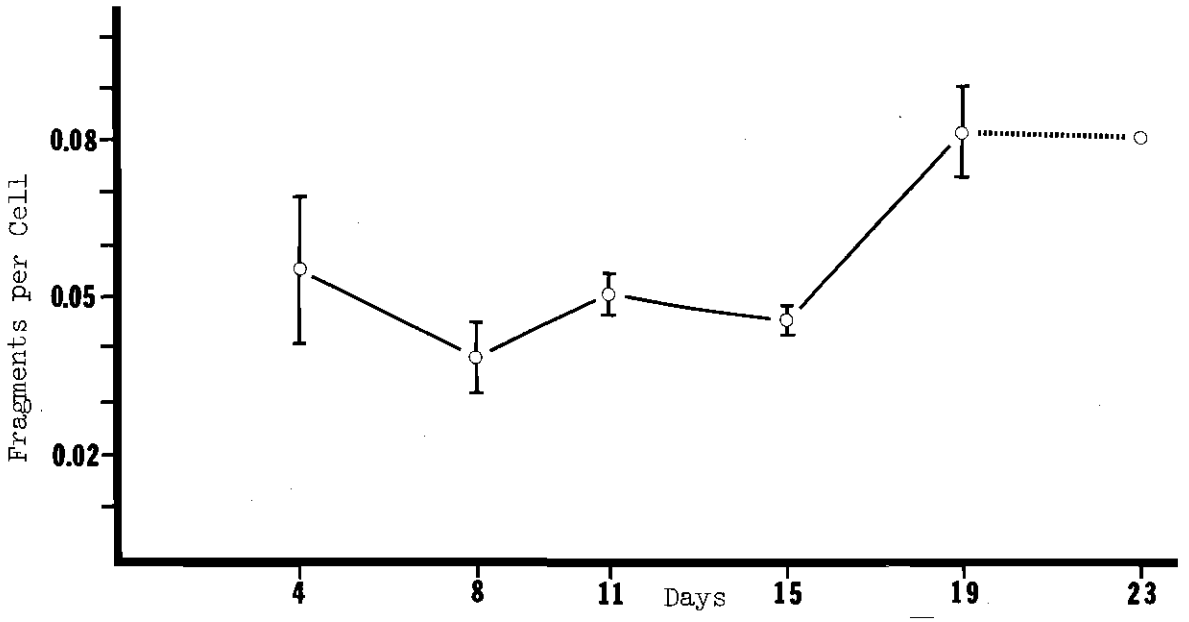

Figure 4. Frequency of Fragments per Cell as a Function of Time in Culture with Standard Error of the Mean Shown.

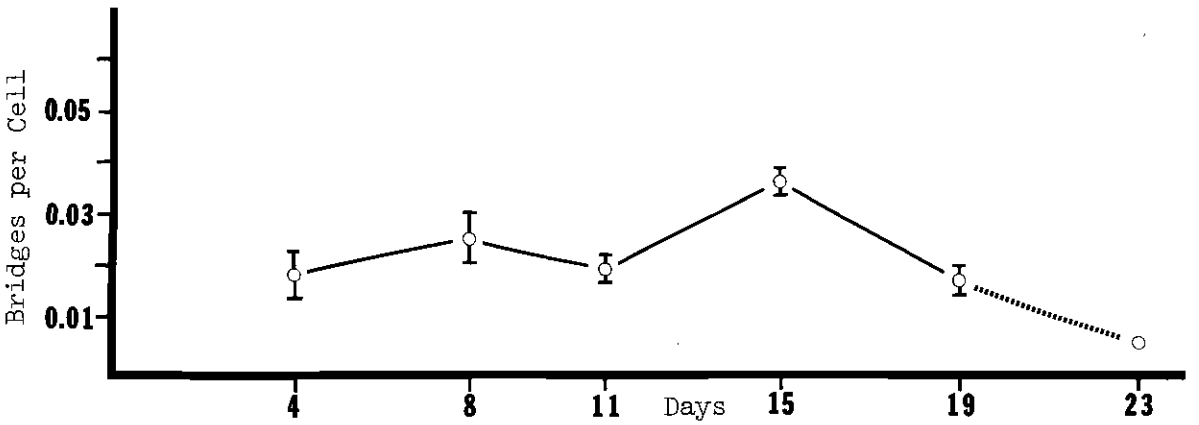

Figure 5. Frequency of Bridges per Cell Plotted as a Function of Time in Culture with Standard Error of the Mean Indicated as Shown.

Table 4. Frequency of Abnormal Anaphases per Cell as a Function of Time in Culture with Standard Errors Shown.

	4 Day	8 Day	11 Day	15 Day	19 Day	23 Day
Number of Cells Analyzed	742	636	653	489	508	200
Abn.Ana./ Cell ± S.E.	0.078± 0.014	0.064± 0.008	0.072± 0.009	0.079± 0.008	0.101± 0.010	0.100

Table 5. Frequency of Fragments Only per Cell with Standard Error Shown.

	4 Day	8 Day	11 Day	15 Day	19 Day	23 Day
Number of Cells Analyzed	742	636	653	489	508	200
Frags/Cell ± S.E.	0.055± 0.021	0.038± 0.010	0.050± 0.006	0.045± 0.004	0.081± 0.013	0.080

Table 6. Frequency of Bridges per Cell with Standard Errors Shown.

	4 Day	8 Day	11 Day	15 Day	19 Day	23 Day
Number of Cells Analyzed	742	636	653	489	508	200
Bridges/ Cell ± S.E.	0.018± 0.006	0.025± 0.007	0.019± 0.003	0.036± 0.004	0.017± 0.004	0.005

appears to be a slight increase in the frequency of abnormal anaphases per cell up through 19 days. However, statistical analysis using the method of Cathcheside, Lea, and Thoday (1946) reveals that there is no significant difference at the 0.05 probability level between the results represented. Figure 4 and Table 5 represent the frequency of deletions, seen as fragments (single and double), found per cell. Statistical analysis reveals no significant difference between the four, eight, eleven, and fifteen day results. However, analysis shows that there is a significant difference at the 0.05 probability level between the fifteen-day and nineteen-day results. A comparison of the nineteen-day results with the data from the first analysis (four-day) show no difference; however, the comparison with the eight, eleven, and fifteen-day results show a significant difference. The standard error for the four-day results is large when compared to the standard errors for the other points. Figure 5 and Table 6 present the frequency of bridges per cell. Statistical analysis reveals that there is no difference between the four, eight, and eleven day results. However, the fifteen-day results are significantly different from both the eleven-day and nineteen-day data. The nineteen-day data are not different from the four, eight, and eleven-day results.

The data represented on the above anaphase figures for 23 days resulted from analysis of only one slide and, thus contained only 200 analyzable cells. The results do not appear to differ significantly from the results found at the other time intervals except in the case of bridges per cell where the frequency is low. Since only one slide was analyzed for this time period, there was not enough data for a calculation of standard error.

CHAPTER IV

DISCUSSION

The karyotypes of primary cultures of some mammalian species have been reported to remain stable *in vitro* and typical of the *in vivo* karyotype for a period of time (Hayflick, 1965; and others). However, the results obtained in this investigation support the findings of others (Puck *et al.*, 1958; Ford and Yerganian, 1958; and others): that the karyotype of the Chinese hamster begins to undergo changes immediately after *in vitro* culture.

The frequency of aneuploidy present in these cultures is not unlike that found by other authors. Ford (1958) found 10 percent aneuploidy in primary Chinese hamster cells after 11 days in culture and 16 percent after 33 days *in vitro*. Tjio and Puck (1958), studying Chinese hamster primary cultures for 5 months, found that the level of aneuploidy remained at approximately 6 percent during their investigation. The results of our investigation indicate that the level of aneuploidy present varies from 11 percent to 16 percent, with an average of approximately 14 percent for the period of study.

This level of variability is not found *in vivo*. Bender and Gooch (1961) studied bone marrow preparations of Chinese hamsters and found no abnormal metaphases present. Ford (1964) offers a general explanation for aneuploidy based on the fact that squash preparations and other techniques used in processing slides for analysis often disrupt the cells, spreading chromosomes over a wide area. This satisfactorily explains

the proportion of cells having a hypodiploid complement. Hyperdiploidy could be explained by assuming that a chromosome from another cell may lie directly above or below a cell under study, thus giving the impression of an extra chromosome. Ford (1964) cites investigation of in vivo mouse metaphases which give a frequency of one hyperdiploid complement per 875 cells. This is explained as being due to somatic nondisjunction. Ford also proposes that a cell containing an extra chromosome would be more viable than one missing a chromosome. Thus, hypodiploid aneuploidy is often an artifact of analysis, whereas hyperdiploid aneuploidy may be an example of evolution of the karyotype. The data presented here indicate that a shift occurred to a higher proportion of hyperdiploid cells as time in culture increased. Hsu and Zenzes (1964) found that Chinese hamster cultures are often hyperdiploid upon the first analysis following initiation of the culture. Ford et al. (1959) noted that hyperdiploidy often resulted from apparent centromeric fracture of a medium-sized metacentric chromosome with two telocentric chromosomes resulting. Since idiograms of metaphases were not made, precise chromosome formulae were not possible. However, observations indicated that centromeric fracture did occur in a small number of cases. More often, however, the hyperdiploid state was due to the presence of an extra small metacentric or telocentric chromosome with the remainder of the complement being typical.

The frequency of polyploidy reported here is not different from that presented by others. These data indicate that an average of four percent of the cells analyzed were polyploid, with about one percent of these triploid cells and the remainder tetraploid. Hsu (1962), using

two adapted cell lines, found an incidence of polyploidy of 5 to 10 percent. Yerganian and Leonard (1961) showed an incidence of polyploidy of from one to 25 percent with the majority being tetraploids resulting from endoreduplication. A comparison of the frequency of tripolar division at anaphase and triploidy at metaphase obtained in this investigation shows good agreement. This could be due to the reduction of chromosome number by a tripolar division on a tetraploid complement. The presence of the tetraploid cells can be explained by failure to complete anaphase or by endoreduplication, both of which are known to occur in vitro.

For detailed analysis of the frequency of chromosome aberrations, metaphase studies are usually undertaken. However, for comparative purposes, anaphase analysis is frequently used. Aberrations found most often at anaphase are fragments, single and double, and bridges, with or without accompanying fragments. The majority of these abnormalities are derived from chromosome or isochromatid breaks followed by sister reunion of the broken chromatid ends, as indicated by equal-armed bridges (Ford et al, 1959). The appearance of fragments accompanying the bridge indicates that breakage and reunion occurred following the previous cell division cycle. Lack of fragments indicates the possibility of the breakage-fusion-breakage cycle of McClintock (1929). Conger (1965), in work with Tradescantia, has found that analysis for abnormal anaphases detects roughly 20 percent of the chromosomal damage actually present and analyzable at metaphase. This is due to bridge fracture and inclusion of fragments in the chromosome masses at the poles.

The frequency of single fragments found at anaphase corresponds

to the frequency of chromatid breaks found at first metaphase after irradiation (Lea, 1955). The results of this investigation indicate that the frequency of fragments found increases significantly after 19-days in vitro. However, the data of the 19 day analysis were found not to be significantly different from that of the first, or 4-day, analysis. This would be explained by the findings of Hsu (1962), who reported that the frequency of chromatid breaks was highest among cells in mitosis during the first days following subcultivation. This frequency diminishes as the culture matures. Hsu also found that the frequency of chromatid breaks increased with age of stock culture. Thus, the results found in this investigation are consistent with the findings of others.

Aberrations that lead to anaphase bridges, both single and double, correspond to the frequency of dicentric and ring chromosomes and can prevent cytokinesis (Wolff, 1968). Levan and Biesele (1958) found an average of 9.1 percent abnormal anaphases including bridges in mouse cultures. Sax and Passano (1961) found in human primary cultures that the percentage of bridges and fragments increased from 3 months to 6 months in vitro with bridges increasing from 0.09 percent at 3 months to 1.9 percent at 6 months. The results of this investigation with Chinese hamster cultures exhibit no rise during the 30-day period. The frequency of bridges per cell did increase significantly at 15 days but was not different from the 4, 8, and 11-day analyses on the 19th day. No explanation is offered for this. The occurrence of some bridges without fragments suggests that a breakage-fusion-breakage cycle may be present (McClintock, 1929). It is possible that the fragments were masked by the polar masses, as noted by Conger (1965) in

Tradescantia. However, the low number and small size of these chromosomes and the relatively wide separation at anaphase in Chinese hamster primary cultures tend to eliminate this possibility.

Chinese hamster primary cultures exhibit a spontaneous aberration frequency comparable to that seen in Phase III cells of human cultures. However, the rate of proliferation in the Chinese hamster primary cultures was found to be high, whereas Phase III of the human cultures is evidenced by a decrease in proliferative ability. No explanation for the response of Chinese hamster primary cultures has been offered.

The level of abnormalities present during the first 30 days in vitro are predictable, with the frequency of fragments which occur spontaneously increasing significantly. The availability of Cricetulus griseus, the ease of handling the animal, the readiness with which the embryonic tissue begins to grow in vitro, and the relatively low level of spontaneous abnormalities render this animal and primary cultures derived from it to be of great cytogenetic value for future research.

CHAPTER V

CONCLUSIONS

A cytogenetic study of embryonic Chinese hamster cells during the first 30 days of *in vitro* culture led to the following observations:

1. The frequency of aneuploidy at metaphase does not increase significantly over the 19-day observation period *in vitro*.

2. The frequency of polyploidy at metaphase does not increase significantly during the 19-day period.

3. The frequency of abnormal anaphases increases during the first 4 weeks *in vitro* due to a significant increase in frequency of fragments per cell at the 19-day analysis.

4. The low frequency of spontaneous aberrations and excellent cytogenetic properties render primary cell cultures of embryonic Chinese hamsters (*Cricetulus griseus*) very satisfactory for detailed cytogenetic research.

CHAPTER VI

RECOMMENDATIONS

Based on insight gained during the course of this investigation, the following recommendations are noted as being of future interest:

1. Detailed study of relative length of chromosomes, centromeric index, and arm ratio should be performed in order to assure that no concealed abnormalities are occurring during the first few weeks *in vitro*.

2. Investigation of change in radiation sensitivity as a function of time in culture should be undertaken.

3. An extension of the present investigation for a greater period of time, with more emphasis on the morphological changes occurring in the chromosomes would be of interest with respect to transformation of a cell strain to a cell line.

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